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Distribution and conservation status of *Speleonycta ozarkensis* (Insecta, Zygentoma, Nicoletiidae) from caves of the Ozark Highlands of Arkansas and Oklahoma, USA

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Abstract

The "thysanuran" (Zygentoma: Nicoletiidae) *Speleonycta ozarkensis* is the only troglobiotic nicoletiid from the Ozark Highlands. It was originally described with only four specimens from four different cave systems in Arkansas and Oklahoma. The scarcity of available specimens has made it difficult to determine whether morphological variation among populations represents intraspecific or interspecific variation. We examined molecular (16S rRNA) variation among populations and found no evidence that they represent a species complex. Because of its limited distribution and lack of ecological and life history data, *S. ozarkensis* may be a species of conservation concern. We therefore conducted a conservation status assessment. We bioinventoried 44 caves in Arkansas and Oklahoma to determine the distribution of *S. ozarkensis.* A new locality in Adair Co., Oklahoma, was discovered and new specimens were collected to better assess morphological variation among populations. Data on ecology and life history was gathered. We determined the conservation status of the species and identified potential threats to existing populations. Despite being known from a few localities, *S. ozarkensis* has a broad distribution approaching 10,000 km^2. Molecular

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data suggest *S. ozarkensis* is capable of considerable dispersal and is primarily an epikarstic species, perhaps explaining why it has been infrequently collected from caves. Conservation assessments revealed that *S. ozarkensis* is at a slight risk of extinction. We identified seven threats impacting populations that vary in scope and severity, but only recreational caving (three caves) and development associated with urbanization (one cave) have the greatest potential to immediately impact populations.

Keywords
Zygentoma, Thysanura, Cubacubaninae, *Speleonycta ozarkensis*, Ozarks, troglobite, cave, 16S rRNA

Introduction

A new genus and species of troglobiotic nicoletiid ("thysanurans", bristletails or silverfish), *Speleonycta ozarkensis* Espinasa et al., 2010 (Insecta: Zygentoma: Nicoletiidae: Cubacubaninae), was recently described from specimens collected from four localities in the Ozarks Highlands of northeastern Oklahoma and northwestern Arkansas. The four localities are Bear Hollow Cave and Chambers Hollow (=Uno) Cave in Benton Co., Arkansas, Single Barrel Cave in Cherokee Co., Oklahoma, and Black Hollow Cave in Delaware Co., Oklahoma. At the time of the description, nicoletiid silverfishes were also known from two additional localities in Arkansas (Tweet's Cave and Wolf Creek Cave in Newton Co.) and one locality in Oklahoma (McGee's Cave in Delaware Co.), but specimens were not available for examination. Espinasa et al. (2010) assumed that all seven populations belonged to the same species, and that it was endemic to cave systems within the Ozark Plateau region, spanning a distribution approaching 10,000 km^2 (Figure 1).

Despite several collecting trips, these caves only yielded a single specimen of *S. ozarkensis* from each locality (Espinasa et al. 2010). The four available specimens at the time of description varied with respect to stage of postembryonic development and gender (2 males and 2 females). The scarcity of specimens has made it difficult to determine whether morphological variation among populations represents intraspecific or interspecific variation. There is the possibility that the reported sexual secondary differences (Espinasa et al. 2010) among specimens of the same gender but from different caves actually reflect that the species is a complex of closely related species. For example, multiple cave populations of nicoletiids of the genus *Texoreddellia* spanning over 300 km of Texan karst were thought to be the same species (*T. texensis*; Wygodzinsky 1973), until molecular analyses revealed this group actually represents a complex of at least six closely related species (Espinasa and Giribet 2009).

Because of the species' limited distribution and lack of information on ecology and life history, *S. ozarkensis* may be a species of conservation concern. However, the conservation status of this nicoletiid has not yet been assessed under NatureServe and IUCN Red List criteria, because of the recency of its description. In addition, genetic information (16S rRNA gene) is only available for a single specimen collected from Chambers Hollow (=Uno) Cave, Benton Co., Arkansas. The objectives of the present study were to: (1) determine the distribution of *S. ozarkensis* within the Ozark Highlands of northwestern

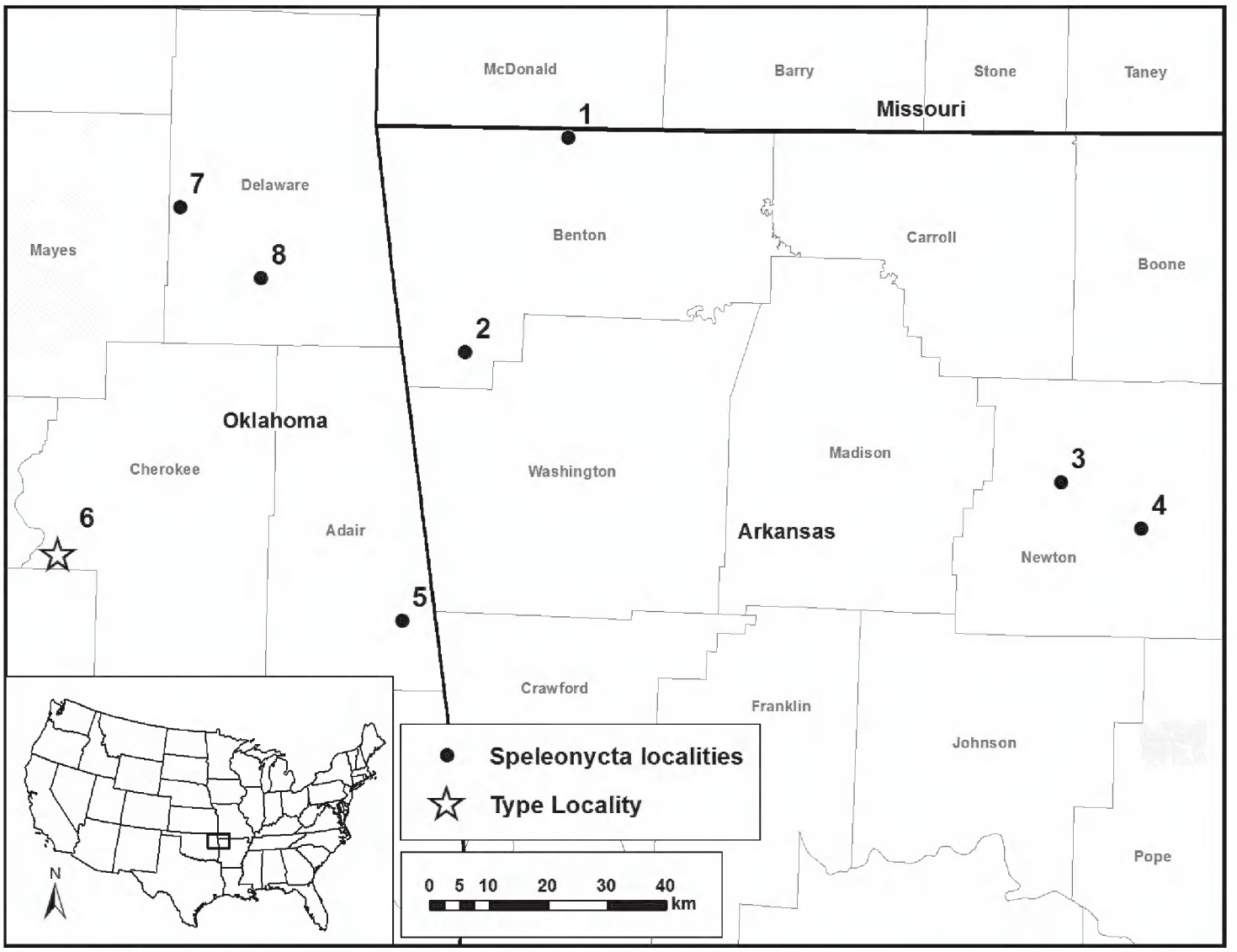

Figure 1. Distribution of *Speleonycta ozarkensis*. Numbered localities correspond to those listed in Table 1.

Arkansas and northeastern Oklahoma; (2) compare newly collected specimens to better assess morphological variation among populations; (3) generate novel DNA sequences for new populations; (4) collect information on ecology and life history, such as microhabitat within caves; and (5) determine the conservation status of *S. ozarkensis* and identify potential threats to existing and newly discovered populations.

Materials and methods

Cave surveys. We surveyed 44 caves in northwestern Arkansas and northeastern Oklahoma within the suspected range of *S. ozarkensis* between January 2010 and May 2014. Sampling effort varied among caves due to variation in cave length and amount of available habitat. Nonetheless, visual encounter surveys (VESs) were conducted by a minimum of two observers for at least two person-hours in all caves and consisted of visually searching terrestrial habitat (cave floor, cave walls, and underneath rocks and other debris) in human-accessible passages. Specimens encountered were collected by hand and deposited into 95% ethanol for preservation. Any rocks, logs and other debris were returned to their original positions to minimize habitat disturbance.

Morphological analysis. Observations of body parts were made with a Motic K series stereo microscope. Specimens in ethanol were observed directly in petri dishes so as to preserve the integrity of the specimens.

Molecular methods. Genomic DNA from two specimens was extracted using Qiagen's DNEasy® Tissue Kit by digesting a leg in lysis buffer. PCR amplification and sequencing of a 501 bp portion of the 16S rRNA mitochondrial gene followed standard protocols and primers used previously for nicoletiids (Espinasa and Giribet 2009). Sequencing reactions were performed using original PCR primers and run on an ABI PRISM 3100 (Applied Biosystems) sequencer. Forward and reverse sequences for each sample were aligned and edited in Sequencher 3.0 with ambiguous base calls verified manually by examining the electropherogram for each sequence. These sequences were aligned to each other into contigs and then a Blast analysis was performed to find which sequence in GenBank had highest similarity.

Conservation assessments. We conducted conservation assessments under both NatureServe and IUCN Red List criteria. NatureServe's system of assessing conservation status uses ten primary factors grouped into three main categories: rarity, trends, and threats (Master et al. 2009). Rarity factors include range extent, area of occupancy (AOO), number of occurrences, number of occurrences with good viability or ecological integrity, population size, and environmental specificity. Trend factors include both short-term and long-term trends in population size, extent of occurrence (EOO), AOO, number of occurrences, and viability or ecological integrity of occurrences. Finally, threat factors include threat impact and intrinsic vulnerability to threats. Other information is often used in addition to the ten conservation status factors to assess conservation status, including the number of protected or managed occurrences, rescue effect, and other considerations. NatureServe conservation global status assessments for each lineage were calculated using default points and weights with the NatureServe Rank Calculator worksheet available in Microsoft Excel (Faber-Langendoen et al. 2009). All *Speleonycta* populations are known from caves, so we considered the species to have a 'very narrow' environmental specificity.

Both NatureServe and IUCN Red List assessments use two different measurements of geographic range size: extent of occurrence (EOO; referred to as range extent by NatureServe) and area of occupancy (AOO). We calculated EOO and AOO using the web-based program GeoCAT (Bachman et al. 2011). EOO was calculated as a convex hull, which is the smallest polygon that contains all the sites of occurrence and no interval angles exceeding 180°. We followed NatureServe (Faber-Langendoen et al. 2009) and IUCN (2010) guidelines and used a grid size of 2 km (4 km^2) to estimate AOO. Accurate estimates of population size are difficult for most subterranean species due to the inaccessibility of and difficulty associated with surveying in cave habitats. Short-term and long-term trends were not used in conservation status assessments because of the lack of historical data for most occurrences. The IUCN-Conservation Measures Partnership Classification of Threats (Salafsky et al. 2008) following the threat assessment protocol outlined in Master et al. (2009) was employed to evaluate the scope, severity and timing of specific threats that are either observed, inferred or suspected to impact *Speleonycta* populations.

A species may be classified as critically endangered (CR), endangered (EN), or vulnerable (VU) on the IUCN Red List if it meets specific conditions under any one of these five criteria (IUCN 2001): (A) past, present, or projected reduction in population size over three generations; (B) small geographic range in combination with fragmentation, population decline or fluctuations; (C) small population size in combination with decline or fluctuations; (D) very small population or very restricted distribution; and (E) a quantitative analysis of extinction risk. Criteria for threat classification under categories A, C, and E require evidence of declining trends in population size. Unfortunately, such data are lacking for *Speleonycta* populations at present. Consequently, our assessments focused on criteria under categories B and D.

Results

Since 2010, we surveyed 14 caves in northwestern Arkansas, including 5 in Benton, 7 in Carroll, one in Newton, and one in Washington counties, and 30 caves in northeastern Oklahoma, including five in Adair, one in Sequoyah, and 24 in Delaware counties. Apart from the seven caves where nicoletiids had previously been reported, at only a new single locality were nicoletiids found. Three nicoletiids were observed at a cave (AD85) in Adair County, Oklahoma, on 18 November 2012 (Fig. 2). The cave consists largely of a maze of largely dry, joint-controlled passages with a few isolated drip pools. All three individuals were observed underneath rocks in a ca. 50 m section of passage. The cave was very dry due to prolonged drought conditions in the region. Presumably, these specimens were seeking refuge underneath larger rocks that retained some moisture. Two specimens were collected for morphological and genetic studies. In addition to *S. ozarkensis*, other species were observed in the same section of the cave, including diplurans (*Litocampa* sp.), harvestmen (*Crosbyella* sp.), and fungus beetles (*Ptomaphagus cavernicola* and *P. shapardi*).

The two specimens collected were adult males measuring 7.3 and 8 mm in body length and were identified as *S. ozarkensis*. Despite considerably smaller than the two males used to described the species by Espinasa et al. (2010), which measured 11 mm, the males collected from AD85 also exhibit adult characters, including: (a) pedicellus with clusters of unicellular glands, and a bladelike spine not very sclerotized and with more unicellular on the outer border; (b) tibia of first leg very stout (two times longer than wide) with a large bulge with three distinctly long, sclerotized, and curved macrochaetae; (c) paramera attain apex of stylets IX, are stout with a distal semi-eversible vesicle and with long specialized macrochaetae; and (d) cerci with sensory pegs.

The 16S rRNA sequences of both specimens collected from AD85 were identical. Moreover, the sequences were also identical to the 16S rRNA sequence from the *S. ozarkensis* specimen previously collected from Chambers Hollow (=Uno) Cave, Benton Co., Arkansas (GenBank no. KJ128288).

Conservation assessment. *Speleonycta ozarkensis* has an EOO of 9,833.69 km^2 and an AOO of just 32 km^2 in the Ozark Highlands of northeast Oklahoma and

Figure 2. *Speleonycta ozarkensis* from AD85, Adair Co., Oklahoma.

northwest Arkansas (Fig. 1). The species has only been documented from eight caves in five counties. Estimates of population size are unknown but believed to be small. The maximum number of individuals observed during a single survey is three from AD85; a single individual has been observed at the other seven localities. This species was unknown to science until 2010. Consequently, inferences on trends in population sizes, quality of habitat and range size are extremely limited. Quality of habitat at the eight known localities has not changed significantly in the last five years.

We identified seven threats documented or suspected to impact populations of *S. ozarkensis* at the present or in the future, including residential and commercial development, agriculture (livestock farming and ranching), energy production and mining, amateur and scientific collecting, recreational caving, dams and water management, and pollution. These threats vary in scope (negligible to restricted), severity (negligible to serious) and overall impact (negligible to low) among populations. The most significant threats include recreational caving at localities with unrestricted access and the potential for urbanization within the recharge zone of Bear Hollow Cave in Benton Co., Arkansas. Of the eight known occurrences of *S. ozarkensis*, the main entrances of at least five caves occur on public lands or are managed for their cave and karst resources. Two caves are located on U.S. Forest Service land in Arkansas and are gated or have restricted access due to the presence of endangered bats (Chambers Hollow and Wolf Creek caves). One cave is located on public land owned by the state of Oklahoma (Black Hollow Cave). Additionally, two caves are owned by The Nature Conservancy and are gated (Bear Hollow and McGee's caves). Scientific and amateur

Table 1. Known localities and specimens of *Speleonycta ozarkensis* in the Ozark Highlands of Arkansas and Oklahoma.

Locality No.	Cave	County	State	Ownership /Management	No. Specimens
1	Bear Hollow Cave	Benton	AR	The Nature Conservancy	1
2	Chambers Hollow Cave	Benton	AR	U.S. Forest Service	1
3	Tweet's Cave	Newton	AR	Private	1
4	Wolf Creek Cave	Newton	AR	U.S. Forest Service	1
5	Wady Cave (AD85)	Adair	OK	Private	3[a]
6*	Single Barrel Cave	Cherokee	OK	Private	1
7	Black Hollow Cave	Delaware	OK	State of Oklahoma	1
8	McGee's Cave	Delaware	OK	The Nature Conservancy	1

* type locality

[a] two specimens vouchered.

collections are believed to have had a very minimal affect on any single population, as only six specimens have been collected to date. We assigned an overall threat impact of "Medium" based on a NatureServe conservation threat assessment.

We assigned a NatureServe conservation global rank of G3 (Vulnerable) to *S. ozarkensis* and state ranks of S2 (Imperiled) and S1S2 (Critically Imperiled to Imperiled) for Arkansas and Oklahoma populations, respectively. We classified *S. ozarkensis* as "Near Threatened" under the IUCN Red List classification. While *S. ozarkensis* classifies as "Vulnerable" under Criterion 1a (EOO < 20,000 km^2 and severely fragmented range or known to exist at no more than 10 locations), there is insufficient evidence at the present time to infer if a continual observed or projected decline (Criterion 1b) and/or extreme fluctuations (Criterion 1c) in EOO, AOO, extent or quality of habitat, number of populations or number of mature individuals exists.

Discussion

Few nicolettid are known from caves in the US despite the family being common in neotropical caves (Espinasa and Giribet 2009). In temperate regions, cave nicoletiids have a limited distribution and are known only from California (Espinasa and Botelho in press), Arizona (*S. anachoretes*; Espinasa et al. 2012), Texas (at least six species in the genus *Texoreddellia*, Espinasa and Giribet 2009) and the Ozark Highlands of Arkansas and Oklahoma (*S. ozarkensis*). *Speleonycta ozarkensis* is the only described nicoletiid thysanuran (silverfish) from the Ozark Highlands.

Despite only being collected from eight caves in five counties, *S. ozarkensis* apparently has a broad distribution approaching 10,000 km^2 and includes three ecoregions (Dissected Springfield Plateau, Lower Boston Mountains and Upper Boston Mountains). Ecoregions are defined by general similarity of surface ecosystems, including similarity in both biotic and abiotic characteristics (such as geology, physiography and hydrology that are important constraints in cave development). While previous studies have shown that

ecoregions often delimit range boundaries for many subterranean organisms (Niemiller and Zigler 2013, Niemiller et al. 2013), this was not the case for *S. ozarkensis* as its broad distribution spans three different ecoregions (Woods et al. 2004; Woods et al. 2005).

More important, molecular data indicate that this species may not be comprised of significant fragmented and isolated populations as distributional data might otherwise suggest. DNA sequences of the 16S rRNA gene were identical between specimens from Chambers Hollow Cave in Benton Co., Arkansas, and AD85 Cave in Adair Co., Oklahoma. These two caves are separated by 48.8 km (straight-line distance). Within the subfamily Cubacubaninae, intrapopulation variation averages 1.7 nucleotide differences (range 0–7; n = 29) while interpopulation variation averages 3.4 nucleotide differences (range 0–13; n = 22) within species at the 16S rRNA locus (Espinasa and Giribet 2009). Among sister species, genetic variation averages 31.2 nucleotide differences (range 10–64; n = 14). Identical gene sequences between the specimens located ca. 50 km away suggests that *S. ozarkensis* is capable of considerable dispersal and is primarily an epikarstic species exploiting the extensive network of cracks, fissures and smaller cavities nearer the surface than larger subterranean voids that are human accessible. This may explain why *S. ozarkensis* has been infrequently collected from caves.

A broad distribution despite few known localities has obvious implications with regards to conservation and management of *S. ozarkensis*. Our conservation status ranks, G3 (Vulnerable) for NatureServe and "Near Threatened" for IUCN Red List, reflect a broad distribution as well as a medium threat impact. While only known from eight cave systems, five *S. ozarkensis* populations are afforded some protection. Four caves are gated and one occurs on public land and is managed for its biological and other resources. Restricted access limits potential negative impacts to the populations from recreational caving. The three other localities occur on private lands and may be at a greater risk from recreational caving. The population at Bear Hollow Cave in Benton Co., Arkansas, faces the greatest risk of impacts associated with urbanization. Although the risk is currently low, we recommend that residential and commercial development within the recharge zone and the potential impacts to cave life within this cave system be monitored over the next 10 years. In addition, another recommendation is to resurvey and monitor known localities while also searching for additional cave systems that may harbor *S. ozarkensis*. The establishment of long-term monitoring programs is needed to provide data on population sizes and threats to individual populations over time. Additional surveys are also warranted to document new localities but also assess whether the distribution of *S. ozarkensis* is greater than currently known. The species likely also occurs within cave systems and epikarst of the Ozark Highlands of southwestern Missouri.

The two specimens collected from AD85 Cave in Adair Co., Oklahoma, have increased our understanding of the postembryonic development of the *S. ozarkensis*. A typical adult morphology and presumably sexual maturity has been attained by 7.3 mm, indicating that *S. ozarkensis* reaches sexual maturity at a length comparable to troglobiotic nicoletiids in the sister genus *Texoreddellia* (Espinasa and Giribet 2009). In addition, when reviewing the morphology of the new specimens from AD85, a

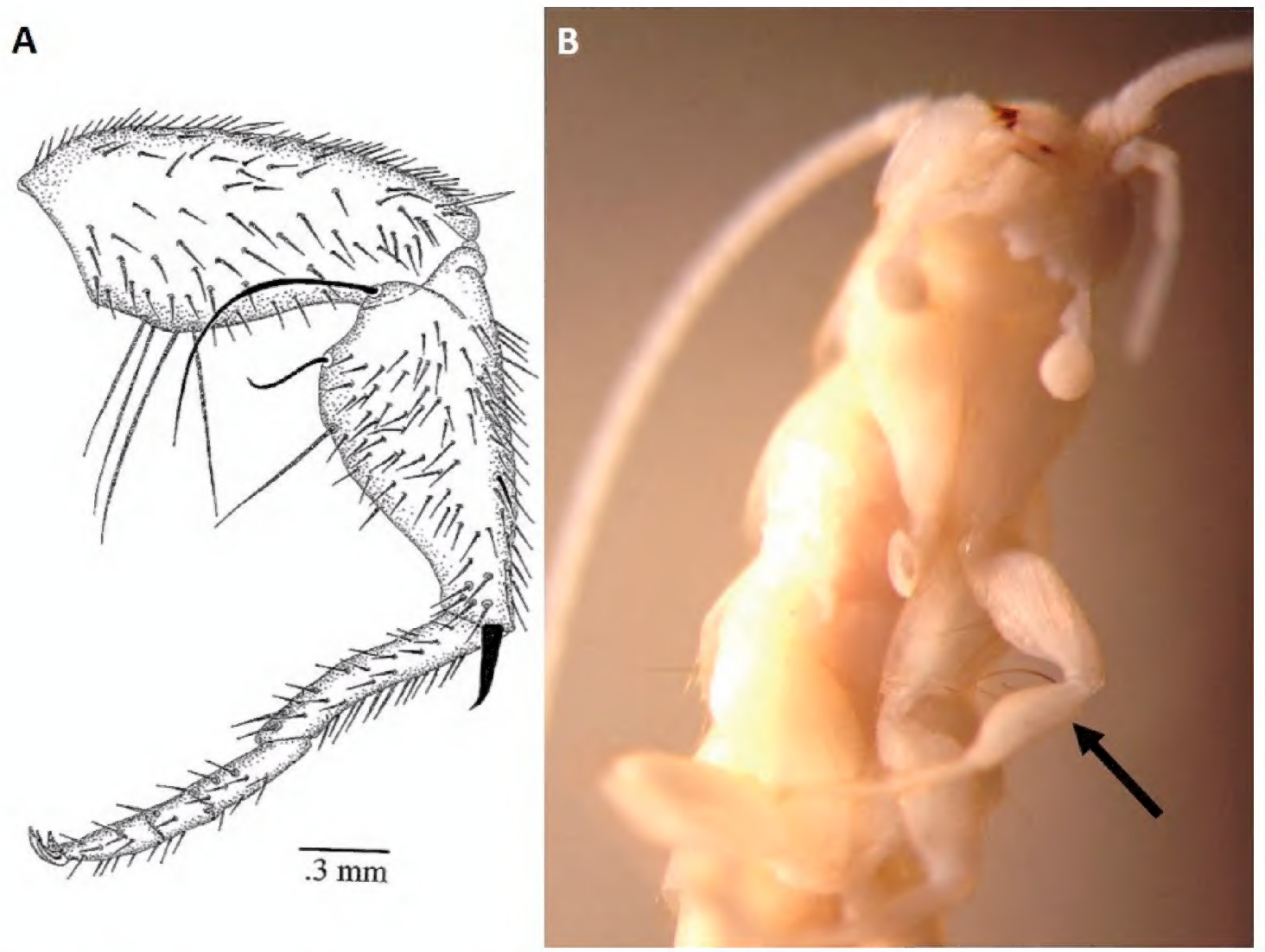

Figure 3. When *Speleonycta ozarkensis* was originally described (Espinasa et al. 2010), the diagnosis states that "tibia of second leg very stout (2 times longer than wide) with a large bulge with 3 distinctly long, sclerotized, and curved macrochaetae", as shown in (**A**). This description was based on a single specimen that had already been dissected and mounted in a fixed slide, with the legs isolated from each other. Examination of new specimens (**B**) shows that the order of legs was misidentified and that the modification is actually on the first leg pair.

mistake in the original description of the species became evident. In the original description it reads, "On male holotype, tibia of *second* leg very stout (two times longer than wide) with a large bulge with three distinctly long, sclerotized, and curved macrochaetae" (Fig. 3A). These structures were described based on a single individual because "the male from Uno Cave had neither (first or) second legs…so it is unknown if the three distinct macrochaetae are present in the legs." The two new specimens from AD85 have these diagnostic modifications, but on the *first* leg (Fig. 3B). Regrettably, when the holotype from Single Barrel Cave in Cherokee Co., Oklahoma, was provided for examination, the specimen had already been dissected and mounted in a fixed slide, with the individual legs separated from each other. The order of the legs was assumed by their position in the slide and their proportional size. It is now evident that the enlarged bulge makes the first leg appear larger than the second leg in this species, thus contributing to its incorrect identification. Diagnosis of the species should now say that modifications of male legs are on the first pair, not on the second.

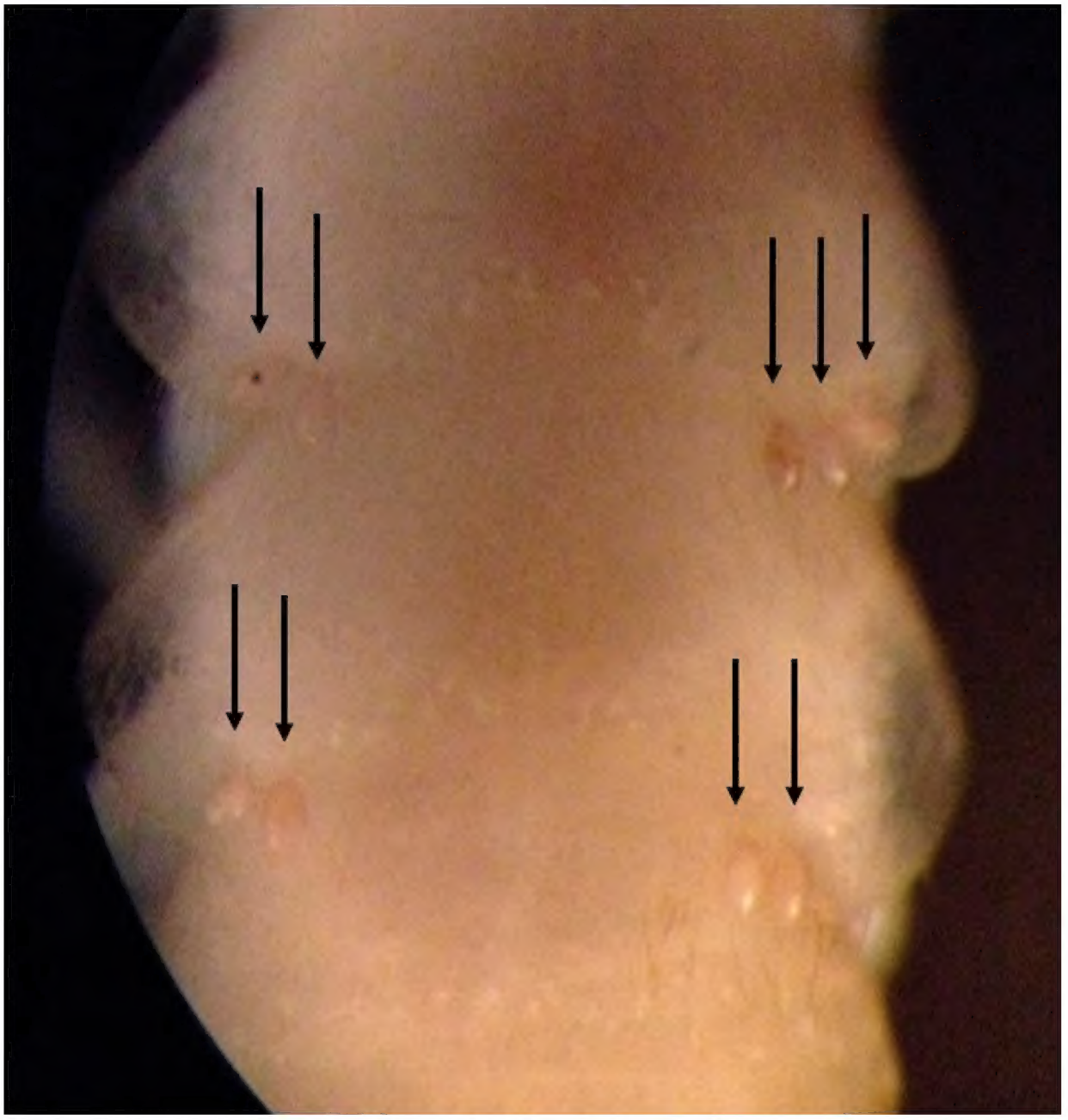

Figure 4. Parasitic acari on thoracic notas of *Speleonycta ozarkensis* from AD85 Cave. They await taxonomic description.

An interesting final observation is that the specimens from AD85 in Adair Co., Oklahoma, are host to parasitic mites (Acari; Fig. 4). Parasitic mites in nicoletiids have also been observed in troglobiotic *Anelpistina mexicana* Espinasa, 1991, but never reported in the literature. These parasitic acari await taxonomic description.

Conclusion

Speleonycta ozarkensis is the only described troglobiotic thysanuran from the Ozark Highlands. New biological inventories since 2010 in Arkansas and Oklahoma yielded only one new locality, AD85 in Adair Co., Oklahoma, bringing the total number of occurrences to eight caves in five counties. Despite being known from a few localities, *S. ozarkensis* has a broad distribution approaching 10,000 km^2. In addition, molecular evidence suggests that *S. ozarkensis* is capable of considerable dispersal and may be primarily an

epikarstic species, perhaps explaining why it has been infrequently collected from caves. Our conservation assessments showed that *S. ozarkensis* is at a slight risk of extinction. We identified seven threats impacting populations that vary in scope and severity, although only recreational caving (three caves) and development associated with urbanization (one cave) have the greatest potential to impact populations in the short term.

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